201-14932B

Substance Group: **Nitric Acid, 2-Ethylhexyl Ester**

Summary prepared by: **Petroleum Additives Panel**
Health & Environmental Research Task Group

1. Environmental Fate and Pathways
1.2. Hydrolysis
Robust Summary 18-Hydro-1

CAS No.	CAS# 27247-96-7
Test Substance Name	Nitric acid, 2-ethylhexyl ester
Method/Guideline	EC Method C7
GLP	Yes
Year	1998
Remarks for Test Conditions	The test substance was dissolved in aqueous media buffered at pH 4, 7 and 9. The concentration of the test substance was determined as a function of time. The logarithms of the concentrations are plotted against time and the slope of the line used to calculate the rate constant. The concentrations in the test solution were determined by a gas chromatographic method using mass spectrometric detection. The test substance was used as the analytical standard. Buffer solutions included: pH 4 – phthalate buffer; pH 7 – phosphate buffer; pH 9 – borate buffer. Buffer solutions were incubated in the water bath at the test temperature and their pH adjusted to the nominal value. The test solution was prepared by adding the test material in methanol to the buffer solution to give a final test material concentration of 6 mg/L and a final methanol concentration of approximately 1%. The study was conducted in borosilicate glass bottles with minimal headspace closed with Teflon lined screw caps. The study was conducted in two phases as follows: Phase I – the test material solution prepared at pH 4, 7 and 9 at 6 mg/L and incubated at 50 ^{o}C. Analysis conducted over several time points. Results obtained at 2.4 hours and 5 days (extrapolated) were used to determine if additional testing was necessary. Phase II - the test material solution prepared at pH 4, 7 and 9 at 6 mg/L and incubated at 25 ^{o}C. Analysis conducted over several time points. This test was run in duplicate. The logs of the concentration of the test material in the different buffers incubated at 50 ^{o}C were plotted against incubation time and fitted to linear regression curves with good coefficients of correlation for each pH. The observed rate constant of the reaction was calculated from the slope for each pH (k_{obs} = slope x 2.303). Reaction half-life was

	calculated ($t_{1/2} = 0.693/k_{obs}$).
Results	The observed rate constants and reaction half lives at 50 and 25 °C were as follows: pH: 4.0 — Rate Constant (50 °C) (min^{-1}): 0.0005657 — Half Life (mins) (50 °C): 1225 — Rate Constant (25 °C) (hour^{-1}): 0.001874, 0.002875 — Half Life (hours) (25 °C): 370, 241 pH: 7.0 — Rate Constant (50 °C) (min^{-1}): 0.0004698 — Half Life (mins) (50 °C): 1475 — Rate Constant (25 °C) (hour^{-1}): 0.004405, 0.008664 — Half Life (hours) (25 °C): 157, 80 pH: 9.0 — Rate Constant (50 °C) (min^{-1}): 0.0004072 — Half Life (mins) (50 °C): 1702 — Rate Constant (25 °C) (hour^{-1}): 0.004695, 0.006441 — Half Life (hours) (25 °C): 148, 108 The duplicate value at pH 7, 25 °C was excluded. The test material was shown to hydrolyze in each of the pH conditions tested following a pseudo-first order reaction. The mean half-life of the hydrolysis reaction at 25°C ranged from 370 (pH 4.0) to 108 hours (pH 9.0).
Conclusions	The test material was shown to hydrolyze in each of the pH conditions tested following a pseudo-first order reaction. The mean half-life of the compound and water reaction at 25°C ranged from 370 hours (pH 4.0) to 108 hours (pH 9.0).
Data Quality	Reliable without restriction (Klimisch Code)
References	Confidential Business Information
Other	Updated: 11/11/2003

2. Ecotoxicity

AQUATIC ORGANISMS

2.1 Acute Toxicity to Fish

Robust Summary 18-Fish Tox –1

Test Substance	
CAS #	27247-96-7
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	Test material purity: 99.9%
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1992).
Test Type	Acute Toxicity to Fish (Static test)
GLP (Y/N)	Y
Year (Study Performed)	1998
Species/Strain	Zebra fish (Danio rerio)
Fish Number	10/concentration
Fish Size	Average length 2.67 cm (2.4-3.0 cm) ; Biological loading 0.3 g/L
Analytical Monitoring	Not performed
Nominal Test Substance Concentration Levels	Control, 1, 10 and 12.6 mg/L (12.6 mg/L is the limit of solubility)
Test Concentration Preparation	Appropriate amounts of the test material were added directly to the experimental water (4 liters). Both the mid and high concentrations were subjected to slight continuous stirring (<20 rpm).
Exposure Period	96 hours
Exposure Conditions	Static (non-renewal test) conditions.
Vehicle	None
Statistical Analysis	None required based on the results.
Dose Rangefinding Study	No
Test Chambers	4-liter tank containing 4 liters of test solution
Diluent Water	Reverse osmosis tap water
Test Solution Water Chemistry During Exposures	Conductivity: 200 umhos/cm Dissolved Oxygen: 100-118% of dissolved air saturation value

	pH: 6.46-6.83 Hardness: 90 mg/L $CaCO_3$
Photoperiod	12-h light per day
Temperature Range	21.1-22.3^{O}C
Positive Control	No
Remarks field for test conditions	Pretreatment: none, fish held for a minimum of 12 days before testing. No feeding 48 hours prior to and during the test. All organisms were observed for mortality and clinical signs of toxicity or abnormal behavior at 2, 24, 48, 72, and 96 hours after initiation of test material exposure.
Results	Cumulative mortality at study termination (96 hours) was as follows: Test Substance Concentration (mg/L) / N / Cumulative Mortality / % Mortality 1.0 / 10 / 3 / 30 10.0 / 10 / 2 / 20 12.6 / 10 / 1 / 10 No undissolved test material was seen on the surface of the test vessels during the study.
Conclusions	The 24, 48, 72 and 96 hour LC50s were >12.6 mg/L.
Data Quality	Reliable with restriction, restriction due to the lack of analytical confirmation of dose concentration.
References	Unpublished confidential business information
Other	Updated: 11/06/2003

2.2 Acute Toxicity to Invertebrates

Robust Summary 18-Daph-1

Test Substance	
CAS #	27247-96-7
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	Test material purity: 99.9%
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984), EEC Directive 92/69-Method C.2 (1992).
Test Type	Static (non-renewal) acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1998
Species/Strain	*Daphnia magna*
Analytical Monitoring	None
Exposure Period (unit)	48 hours
Statistical methods	None required based on results.
Remarks field for test conditions (fill as applicable)	Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Appropriate amounts of the test material were added directly to the dilution water and stirred for 10 to 14 minutes. Twenty daphnids, less than 24 hours old were distributed into each concentration (50 mL/chamber)(5 daphnids/replicate). Daphnids were not fed during exposure. Control test chambers were handled in an identical fashion. Light cycles were maintained at 12-hours of light per day. Test solutions were maintained at 19.3-19.9°C. Dilution water was prepared according to the guideline and contained 200 mL demineralized water and 800 mL natural water.
Test Concentrations	Control, 1, 10 and 12.6 mg/L (12.6 mg/L is the limit of solubility)
Results	
Remarks	Water chemistry: Dissolved oxygen: 8.4 – 8.7 mg/L; pH: 7.65 – 7.80 100% survival occurred in all control, 1 and 10 mg/L test vessels. At 12.6 mg/L 30% immobilization was observed at 24 hours and 20% immobilization was noted at 48 hours. The 24 and 48-hour EC50 values were both >12.6 mg/L. The 24 and 48 hour NOEC was 10 mg/L.

Conclusions	The 24 and 48-hour EC50s were >12.6 mg/L. The 24 and 48 hour NOEC was 10 mg/L.
Data Quality	Reliable with restriction, restriction due to the lack of analytical confirmation of dose concentration.
References	Unpublished confidential business information
Other	Updated: 11/07/2003

2.2 Acute Toxicity to Algae

Robust Summary 18-ALG-1

Test Substance	
CAS #	27247-96-7
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	Test material purity: 99.9%
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #201 Alga Growth Inhibition Test (1984); EEC Directive 92/69 Method C.3 (1992)
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1998
Species/Strain	*Selenastrum capricornutum* (CCAP 278/4)
Element basis (# of cells/mL)	Approximately 10,000 cells/mL
Exposure period/duration	72 hours
Analytical monitoring	None
Statistical methods	The area under the growth curve and the average specific growth rate were determined.
Remarks field for test conditions (fill as applicable)	Individual test concentrations were prepared for each test level. A measured volume of test material was added to a measured volume of test media and stirred for 10 to 18 minutes. A 72-hour sealed static test was carried out in 250 mL Erlenmeyer flasks filled with 50 mL of test solution. Three flasks were prepared for each test concentration and the control. Control flasks containing algal growth medium only. Test chambers were sealed and incubated. The flasks were shaken throughout the study. The pH was determined at time 0 and at 72 hours. Air temperature in the test incubator was monitored throughout the study. Cell counts were made at the start of the study and then at approximately 24-hour intervals. PH was determined for each culture at study start and at approximately 72 hours. Test Levels: Control, 1.0, 10 and 12.5 mg/L (limit of solubility).
Results	The 72-hour No Observed Effect Concentration, based on growth rate and growth inhibition, was 12.6 mg/L, the highest concentration tested.
Remarks	The effective initial concentrations, which induce 50% inhibition as, determined by comparison of area under the growth curve and comparison of growth rates were determined. No significant cell growth or growth rate inhibition was recorded during the 72-hour test period up to 12.6 mg/L of test substance. The no observed effect concentration was defined as the concentration of test substance,

	which induced less than 25% inhibition. The 72-hour loading rates which resulted in 50% reduction in culture growth based on areas under the growth curves and average specific growth rates were both >12.6 mg/L. The highest No Observed Effect Level was 12.6 mg/L . Test Substance Concentration (mg/L) / 72 Hour % Inhibition Cell Growth / 72 Hour % Inhibition Growth Rate 0 / - / - 1.0 / 20 / 6 10.0 / 14 / 2 12.6 / 0 / 0 pH range 7.05-7.20
Conclusions	The 72-hour No Observed Effect Concentration, based on growth rate and growth inhibition, was 12.6 mg/L, the highest concentration tested.
Data Quality	Reliable with restriction, restriction due to the lack of analytical confirmation of dose concentration.
References	Confidential business information.
Other	Updated: 10/31/2003

3.0 Toxicity

3.1 Acute Toxicity

3.1.1 Acute Oral Toxicity

Robust Summary 18-Acute Oral -1

Test Substance	
CAS #	CAS# 27247-96-7
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rats/ Sprague-Dawley strain
Sex	Male/Female
No. of animals/dose	5/sex
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	10 mL/kg
Control group included	No
Remarks field for test conditions	A single dose of the test material was administered intragastrically to five male and five female rats. The animals were observed for signs of toxicity during a 14-day observation period. All surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals after 14 days.
Results	LD50 >10 mL/kg (males and females)
Remarks	Two males and one female died during the observation period. No other signs of toxicity were noted. There were no gross lesions observed during necropsy.
Conclusions	The test article, when administered to male and female Sprague-Dawley rats, had an acute oral LD50 of >10 mL/kg.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 6/05/2003

3.1.2 Acute Dermal Toxicity

Robust Summary 18-Acute Dermal-1

Test Substance	
CAS #	CAS# 27247-96-7
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	Similar to OECD Guideline 402; FHSA Section 191.12
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rabbits/Albino
Sex	Not specified
No. of animals	4
Vehicle	None
Route of administration	Dermal
Dose level	5 mL/kg
Control group included	No
Remarks field for test conditions	This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that the skin of all treated animals was abraded prior to dosing. In addition the guideline calls for the evaluation of five males and five females using at least one dose level. This study was conducted using two males and two females. Given the high dose level tested during this study and the lack of any mortality, these deviations were not considered sufficient to disqualify this study. Prior to topical application of the test material, the hair on the abdomen of each animal was closely clipped. The skin of all treated animals was abraded prior to dosing. A single dose of 5 mL/kg of the undiluted test material was administered dermally to the abraded skin of all animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a rubber dam. The application site was washed with warm water and wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for 14 days after treatment.
Results	LD50 > 5 mL/kg
Remarks	All animals survived the duration of the study. No signs of toxicity were observed.

Conclusions	The test article, when administered dermally as received to four albino rabbits had an acute dermal LD50 of greater than 5 mL/kg.
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the fact that the study design differs from the referenced guideline. However given the high dose level tested (5 mL/kg) and the lack of mortality the study was considered valid and appropriate for review.
References	Unpublished confidential business information
Other	Updated: 6/05/2003

3.3 Repeated Dose Toxicity

Robust Summary 18-Repeat Dose-1

Test Substance	
CAS #	***CAS# 27247-96-7***
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	>99%
Method	
Method/Guideline followed	Similar to OECD 412
Test Type	14-day inhalation toxicity studies in rats with 2 week recovery periods
GLP (Y/N)	Not Specified
Year (Study Reported)	1987
Species	***Rat***
Strain	***Sprague-Dawley CD, 8 weeks of age at initiation of treatment***
Route of administration	inhalation, nose only exposure
Duration of exposure	6 hours/day
Doses/concentration levels	0, 14, 42, 150 ppm (Study I) 0 (unexposed), 0 (chamber room air exposed), 4.3, 42, 420 ppm (Study II)
Sex	Male
Frequency of treatment	5 days/week for 2 weeks
Control and treatment groups	10 male rats/group (5/group sacrificed after 10^{th} exposure; 5/group held for 2 week recovery period) (Study I) 10 male rats/group (5/group sacrificed after 10^{th} exposure; 5/group held for 2 week recovery period) (Study II)
Post exposure recovery period	2 Weeks
Statistical methods	Methods not specified.
Dose rangefinding study	No
Remarks field for test conditions	Treated animals were exposed to the test material as mixed aerosol and vapor atmospheres generated by passing dry nitrogen through midget impingers containing the test material at the low and intermediate levels and by nebulization at the high level. Heated water baths were used to promote volatilization of the test material in the impingers and heating tape was used to minimize condensation of vapors in the transfer tubes. Vapors or aerosols were mixed with air prior to entry into the 150 L stainless steel exposure chambers. Chamber housed control animals were exposed to room air only. In the second study a control group of 5 unexposed rats was included in order to evaluate the effects of restraint and fasting on hepatic vacuolation. Chamber exposure concentrations were measured by gas chromatography with flame ionization detection at 60-minute intervals. Rats were weighed and observed daily. In Study I overnight urine samples were collected from each rat after the 9^{th} exposure. After the 10^{th} exposure, blood samples were collected from each rat for clinical chemistry and hematology analysis. Five rats from each group were

	sacrificed for pathological evaluation. Select organs were weighed. After the 14-day recovery period the remaining rats were subjected to the same clinical pathology and microscopic evaluations. Select organs were weighed. A range of tissues was examined microscopically. In the second study similar procedures were followed except clinical pathology parameters were not evaluated and pathology examinations were limited to the liver and kidneys only.
Results	
Remarks	**Study I** All animals survived until their intended sacrifice. After the 10th exposure, rats from the 150 ppm group exhibited increased hemoglobin and hematocrit values and increased erythrocyte and platelet counts. Mean absolute and relative liver weights were increased compared to controls. Lipid like cytoplasmic inclusions were found in hepatocytes and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. Following the two-week recovery period mean absolute and relative spleen weights were decreased, clinical pathology was normal and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. After ten exposures at 42 ppm mean absolute and relative liver weights were increased compared to controls. Lipid like cytoplasmic inclusions were found in hepatocytes and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. Following the two-week recovery period mean relative spleen weights were decreased, clinical pathology was normal and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. After ten exposures at 14 ppm mean absolute liver weights were increased compared to controls. White blood cell counts were elevated and lipid like cytoplasmic inclusions were found in hepatocytes and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. Following the two-week recovery period mean organ weights were unremarkable, clinical pathology was normal and eosinophilic cytoplasmic inclusions were found in cells of the renal proximal tubules. **Study II** All animals survived until their intended sacrifice. After the 10th exposure, rats from the 420 ppm group exhibited increased mean absolute and relative liver weights compared to controls. A slight loss of cytoplasmic basophilia in hepatocytes and eosinophilic cytoplasmic inclusions in cells of the renal proximal tubule were noted. Lipid like cytoplasmic inclusions were found in hepatocytes, however the same incidence of inclusions was found in the control. Microscopic evaluations were normal following recovery. At 42 and 4.2 ppm, lipid like cytoplasmic inclusions were found in hepatocytes, however the same incidence of inclusions was found in the control. Microscopic evaluations were normal following recovery.

Conclusions	Under the conditions of this study inhalation exposure to this test material resulted in an elevation of mean liver weights at 14 ppm and greater. Lipid like cytoplasmic inclusions were found in hepatocytes at all concentrations tested however since unrestrained, nonfasted rats did not exhibit a similar finding the inclusion bodies were considered a physiological response to restraint and were not considered exposure related. At 14 ppm and greater the test material exposures were associated with eosinophilic cytoplasmic inclusions in cells of the renal proximal tubule. This is a common finding in male CD rats and was attributed to protein absorption from the glomerular filtrate. This finding was less severe after recovery and was not considered biologically significant at concentrations of 42 ppm or less. A treatment related polycythemia was observed at 150. The Study Director concluded that the no observed effect level was 42 ppm.
Data Quality	Reliable with restriction (Klimisch Code) Restriction due to the fact that this summary was prepared based on a study abstract and poster presentation. Individual data were not available.
References	The Toxicologist (7) 202; 1987 and poster presented at 1987 Annual Society of Toxicology Meeting
Other	Updated: 6/10/2003

Robust Summary 18-Repeat Dose-2

Test Substance	
CAS #	CAS# 27247-96-7
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	Federal Register, Volume 43, Number 163 (163.82-2, Subchronic 21 Day Dermal Toxicity Study)
Test Type	21-day dermal toxicity study in rabbits
GLP (Y/N)	Not Specified
Year (Study Performed)	1981
Species	Rabbit
Strain	Albino White (approximately 2-2.6 kg in body weight at initiation)
Route of administration	Dermal, 5 days/week, to the clipped, abraded and unabraded, dorsal surface.
Duration of test	15 days of treatment
Doses/concentration levels	0, 50 and 500 mg/kg
Vehicle control	No
Sex	Males and females
Frequency of treatment	Once/day, 5 days/week for a total of 15 doses.
Control and treatment groups	Three intact and three abraded male and female rabbits in the control group and in both treated groups. An untreated control group was included in the study.
Post exposure observation period	None
Statistical methods	Body weight, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included ANOVA with a Newman Keuls test.
Remarks field for test conditions	The test material was applied to the clipped, abraded or unabraded dorsal surface of the rabbits for 5 days/week for 15 days. Elizabethan collars were used to prevent ingestion. The hair was clipped and shaved from each animal as necessary. The exposed skin of half of the animals was abraded once/week throughout the study. The test material was applied over the clipped area and covered with gauze patches secured in place with surgical hypoallergenic adhesive tape. The trunk of each animal was then wrapped with an impervious material held in place with an elastic bandage. Control animals were handled in an identical manner. After 6 hours the treated areas were wiped gently with corn oil. Clinical observations were made daily. Dermal responses were evaluated daily on dosing days approximately one hour after the completion of the exposure period. Body weight was recorded twice weekly during treatment. Food consumption was estimated every 3 to 4 days during the study. Hematology and clinical chemistry parameters were evaluated pretest and at termination of treatment. Macroscopic examinations were performed on all animals. Select organs were weighed. Microscopic examinations were conducted for all animals. The following tissues were evaluated: treated and untreated skin, gross lesions, brain, heart, liver, spleen, kidneys, urinary

	bladder, ovaries, testes, adrenals, thyroid, stomach, mesentery, small and large intestine and cecum.
Results	
Remarks	Two animals died prior to study termination. These included one high dose female (on day 5) and one low dose male (on day 21). The low dose death was attributed to pneumonia. The cause of the high dose death was not determined. All of the remaining control and treated animals survived the duration of the study. Dermal irritation was observed in both test material treated groups. Erythema and eschar formation and edema ranging from well defined to severe were observed in males and females at both dose levels at both intact and abraded dose sites. Findings in the high dose males were more severe than in the low dose males. A dose response was not clearly evident in the females. No significant dermal findings were observed microscopically. There were no consistent differences exhibited in mean body weight between the treated and control animals. Food consumption was generally unremarkable in all groups throughout the study. The clinical laboratory, organ weight and microscopic data were also generally unremarkable. Based on the in life dermal findings observed in the low dose males and females, a no observed adverse effect level for local effects was established.
Conclusions	Based upon systemic toxicity, a NOEL of >500 mg/kg was established for this study.
Data Quality	Reliable with restriction (Klimisch Code) Restriction due to the lack of correlation between in life dermal findings and microscopic findings of the skin.
References	Unpublished confidential business information
Other	Updated: 12/2/2003

Robust Summary 18-Repeat Dose-3

Test Substance	
CAS #	CAS# 27247-96-7
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	100%
Method	
Method/Guideline followed	Japanese Guidelines for Screening Toxicity Testing Chemicals (Notifications Kanpogyo No. 39, Yakuhatsu No. 229 Kikyoku No. 85 (1984)
Test Type	28-day oral toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1989
Species	Rat
Strain	SD[Crj: CD(SD), SPF], 4 weeks of age at receipt
Route of administration	Oral gavage (syringe and dosing tube)
Duration of test	28 days of treatment plus 14 days of recovery
Doses/concentration levels (dose volume)	0, 20, 100 and 500 mg/kg/day (10 ml/kg)
Vehicle	0.5% Tween 80
Sex	Males and females
Exposure period	28-day treatment duration
Frequency of treatment	7 days/week
Number of animals/sex/group	6 rats/sex/group for 28 day sacrifice 6 rats/sex in control and high dose for 14 day recovery period
Post exposure observation period	14 days in control and high dose groups
Chemical Analysis	Chemical analysis of dosing solutions was conducted by the sponsor and confirmed dosing suspensions were stable for 10 days.
Statistical methods	Student's t-test, Welch's t-test, Armitage's chi square test.
Dose rangefinding study	Yes (acute study and 10 day toxicity study)
Remarks field for test conditions	Single oral doses were administered for 28 consecutive days using a gavage needle. Clinical observations were performed daily. Body weights were recorded prior to the first dose and weekly thereafter. Food consumption was measured weekly. Hematology, clinical chemistry and urinalysis determinations were conducted prior to the 28 day and recovery sacrifices for all survivors. Macroscopic examinations were performed on all animals. The brain, liver, kidney, adrenal, testis and ovary were weighed. A range of tissues was examined microscopically. These included the heart, liver, spleen, kidney and adrenals from all control and high dose animals, the kidney from all low and mid dose animals sacrificed at 28 days and gross lesions from all groups. The kidneys were examined for all animals at recovery.
Results	
Remarks	All of the treated animals survived the duration of the study. Post dosing salivation was observed in the high dose males and females

	during the second week of study and thereafter. Predosing salivation was also observed in the high dose females. Salivation was not observed during recovery. A statistically significant decrease was observed in the mean body weight of the high dose females during the last week of treatment and during the first week of recovery. A statistically significant decrease was observed in the mean food consumption of the high dose females during the last week of treatment but not during recovery. Following 28 days of treatment the high dose males exhibited a statistically significant increase in mean platelet number. No other treatment related changes were observed in the hematology data. The high dose females exhibited a significant increase in urea nitrogen and a significant decrease in chloride following 28 days of treatment but not following recovery. A number of alterations were observed in the urinalysis data of mid and high dose 28 day sacrifice animals, these included: a significantly acidic urinary pH in high dose males and females; a significant increase in protein, ketone bodies, urobilinogen, volume, potassium and chloride in high dose males and females; a significant increase in ketone bodies in the mid dose females; and a significant increase of epithelial cells in the urine sediment of the mid and high dose females. In the high dose recovery females significant decreases in sodium, potassium and chloride were observed. Following 28 days of treatment significant increases were observed in the absolute and relative liver and kidney weights of the high dose males and in the relative liver and kidney weights of the high dose females. Increased relative adrenal weights were also observed in the high dose females. Following recovery relative kidney weights were increased in the high dose males. At the 28 day necropsy enlarged livers were observed in the mid dose males and high dose males and females. Enlargement of the kidneys was observed in the high dose males. Following recovery enlarged kidney was observed in one high dose male. Microscopic changes observed in the kidney of the mid and high dose males included the appearance of hyaline droplets in the proximal tubular epithelium and regenerative changes of the renal tubules. The regenerative change was also observed in one high dose recovery male. None of these effects were observed in the females. There were no significant histological effects in the liver.

Conclusions	The Study Director concluded that the no observed effect level for systemic toxicity was 20 mg/kg/day.
Data Quality	Reliable with restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/17/2003

Genetic Toxicity:

Robust Summary 18-Gentox:-1

Test Substance	
CAS #	CAS# 27247-96-7
Chemical Name	Nitric acid, 2-ethylhexyl ester
Remarks	Test material purity not specified.
Method	
Method/Guideline followed	Similar to OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	N
Year (Study Performed)	1978
Test System	*Salmonella typhimurium*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.1% solution (v/v) in DMSO: 0.001, 0.005, 0.01, 0.05, 0.1ul/plate
Metabolic Activation	With and without (0.5 ml of S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Dimethylsulfoxide (DMSO)
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 Aflatoxin B1 1.0 ug/plate TA98 -S9 2-nitroflourene 5.0 ug/plate TA100 +S9 Aflatoxin B1 1.0 ug/plate TA100 -S9 N-methyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate TA1535 +S9 2-aminoanthracene 5.0 ug/plate TA1535 -S9 N-methyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate TA1537 +S9 2-aminochrysene 1.0 ug/plate TA1537 -S9 9-aminoacridine 100 ug/plate TA1538 +S9 2-aminofluorene 2.0 ug/plate TA1538 -S9 2-nitroflourene 5.0 ug/plate
Vehicle Control	Dimethylsulfoxide (DMSO) 100 ul/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point. Linear regression analysis was used to compute

	the best-fit line of dose response.
Dose Rangefinding Study	No
S9 Optimization Study	Yes
Remarks field for test conditions	This study was conducted in 1978, prior to the adoption of OECD Test Guideline 471. In addition to the tester strains used during this study, the OECD Guideline suggests the inclusion of tester strains *E.coli* WP2 uvrA, or WP2 uvrA (pKM101) or *Salmonella typhimurium* TA102. This study included the use of tester strain TA1538. OECD 471 does not incorporate this strain. These deviations from the test guideline are not considered major study deficiencies. In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle control, and a positive control. Three plates/dose group/strain/treatment set were evaluated. Test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, and S9 mix (if needed). This was overlaid onto the surface of supplemented Noble's agar in a screw-capped tube. Tubes were mixed and poured over a base plate of Spizzizen's minimal medium. Plates were incubated for 48 hours at 37^{o}C.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	In this mutagenicity assay all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response. For each strain, the numbers of revertant colonies in negative control plates were within acceptable limits as defined by historical control data for spontaneous revertants. Sterility controls were negative.
Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/06/2003